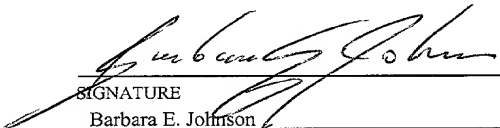


Form PTO-1390 (REV 10-95)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER <b>702-010166</b>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (if known, rec'd 37 CFR 1.5) <b>09/762779</b>
INTERNATIONAL APPLICATION NO. <b>PCT/NL99/00504</b>	INTERNATIONAL FILING DATE <b>06.08.99 (August 6, 1999)</b>	PRIORITY DATES CLAIMED <b>14.08.98 (August 14, 1998)</b>	
TITLE OF INVENTION <b>DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS AND PROCESS UTILIZING SUCH DEVICE</b>			
APPLICANT(S) FOR DO/EO/US <b>Johannes G. A. TERLINGEN and Gerardus H. M. ENGBERS</b>			
<p>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <li><input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. 371.</li> <li><input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</li> <li><input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</li> <li><input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))             <ol style="list-style-type: none"> <li><input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li><input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</li> </ol> </li> <li><input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2))</li> <li><input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))             <ol style="list-style-type: none"> <li><input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li><input type="checkbox"/> have been transmitted by the International Bureau</li> <li><input type="checkbox"/> have not been made, however, the time limit for making such amendments has NOT expired.</li> <li><input checked="" type="checkbox"/> have not been made and will not be made</li> </ol> </li> <li><input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</li> <li><input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</li> <li><input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</li> </ol> <p><b>Items 11. to 16. below concern document(s) or information included:</b></p> <ol style="list-style-type: none"> <li><input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98</li> <li><input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</li> <li><input checked="" type="checkbox"/> A FIRST preliminary amendment.              <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> <li><input type="checkbox"/> A substitute specification.</li> <li><input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li><input checked="" type="checkbox"/> Other items or information             <ol style="list-style-type: none"> <li>WO 00/10012-Front Page with Abstract, specification claims and drawing (23 pp.)</li> <li>Search Report (3 pp.)</li> <li>International Preliminary Examination Report With Annex (7 pp.)</li> </ol> </li> </ol>			

U.S. APPLICATION NO. (If known, see 37 CFR 1.53) <b>09/762779</b>		INTERNATIONAL APPLICATION NO. PCT/NL99/00504		ATTORNEY'S DOCKET NUMBER 702-010166	
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):</b> Search Report has been prepared by the EPO or JPO..... \$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) ..... \$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))..... \$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$1000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$100.00  <div style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b></div>				CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e))				\$ 130.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	24 - 20	4	X \$18.00	\$ 72.00	
Independent claims	4 - 3 =	1	X \$80.00	\$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$ 0.00	
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 1142.00	
Reduction of 1/2 for filing by small entity, if applicable. Small Entity Statement verified by Applicant(s) attorney.				\$ 0.00	
<b>SUBTOTAL =</b>				\$ 1142.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 0.00	
<b>TOTAL NATIONAL FEE =</b>				\$ 1142.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$ 0.00	
<b>TOTAL FEES ENCLOSED =</b>				\$ 1142.00	
				Amount to be: refunded	\$
				charged	\$
a. <input checked="" type="checkbox"/> A check in the amount of \$ <b>1142.00</b> to cover the above fees is enclosed b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Assistant Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>23-0650</u> . A duplicate copy of this sheet is enclosed.  <b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed          and granted to restore the application to pending status.</b>  SEND ALL CORRESPONDENCE TO: Barbara E. Johnson 700 Koppers Building 436 Seventh Avenue Pittsburgh, Pennsylvania 15219-1818 Telephone: (412) 471-8815 Facsimile: (412) 471-4094					
 SIGNATURE Barbara E. Johnson NAME 31,198 REGISTRATION NUMBER					

PATENT APPLICATION/PCT  
Attorney Docket No. 702-010166

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of :

Johannes G. A. TERLINGEN :  
Gerardus H. M. ENGBERS :  
DEVICE FOR INVESTIGATING  
CHEMICAL INTERACTIONS AND  
PROCESS UTILIZING SUCH DEVICE

International Application :  
No. PCT/NL99/00504 :

International Filing Date :  
6 August 1999 :

Priority Dates Claimed :  
14 August 1998 :

Serial No. Not Yet Assigned :

Filed Concurrently Herewith :

Pittsburgh, Pennsylvania  
February 13, 2001

**PRELIMINARY AMENDMENT**

**BOX PCT**

Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

Prior to initial examination, please amend the above-identified patent application

as follows:

**IN THE SPECIFICATION:**

Page 1, after the title, insert the following headings:

**--BACKGROUND OF THE INVENTION**

1. **Field of the Invention--.**

Page 1, after line 8, insert the following heading:

--2. Description of the Related Art--.

Page 2, after line 17, insert the following heading:

--SUMMARY OF THE INVENTION--.

Page 4, after line 38, insert the following heading and paragraph:

--BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 is a plot of observed response with respect to time in the immobilization of albumins onto a COOH disk.--.

Page 5, before line 1, insert the following heading:

--DESCRIPTION OF THE PREFERRED EMBODIMENTS--.

IN THE CLAIMS:

Original claims 1 and 9 were amended during Chapter II proceedings by substituting new claims 1 and 9 in a letter dated October 20, 2000. Please cancel original claims 1-24 and cancel amended claims 1 and 9 and rewrite them as new claims 25-48 as follows.

--25. A device for investigating reactions between interactive chemical or biological species, said device comprising:

a substrate; and

a plasma layer deposited on the substrate, wherein the substrate in turn comprises

5 a film of free electron metal consisting essentially of gold, and wherein the plasma layer deposited on the film of free electron metal comprises sulphur.

26. The device according to claim 25, wherein the plasma deposited layer is supported on a substrate.

27. The device according to claim 25, further comprising a film of a free electron metal.

28. The device according to claim 27, wherein the plasma deposited layer is arranged directly on the free electron metal film and further wherein said electron free metal is selected from the group consisting of copper, silver, aluminum and gold.

29. The device according to claim 25, wherein the plasma deposited layer, comprises one or more chemical or biological functional groups.

30. The device according to claim 29, further comprising one or more wet chemically deposited layer(s), arranged on the plasma deposited layer.

31. The device according to claim 25, wherein the plasma layers comprise one or more compounds selected from the group consisting of amine compounds, sulphur-containing compounds, thiols, sulfides, disulfides and diallyl sulfide.

32. The device according to claim 31, wherein the substrate consists essentially of gold.

33. A process for providing a device for investigating reactions between interactive chemical and biological species, said process comprising the steps of (a) providing a pre-selected substrate, which substrate in turn comprises a film of free electron metal consisting essentially of gold and (b) arranging a layer on the gold film by plasma deposition, which layer  
5 comprises sulphur.

34. The process according to claim 33, wherein the plasma layer is directly deposited onto the substrate or onto a metal film arranged on the substrate.

35. The process according to claim 33, wherein plasma is deposited from a monomer/oligomer/polymer in gas form, preferably being a monomer, said monomer being saturated, partially saturated or unsaturated.

36. The process according to claim 33, wherein the substrate is subjected to a pre-cleaning step comprising pre-treating the substrate by means of a plasma etching step before the plasma deposition step.

37. The process according to claim 33, wherein the gas plasma is deposited under the following conditions:

a discharge power of up to 5000 W;

an exposure duration of up to 1000 s;

- 5
- a plasma gas flow of up to 10000 cm<sup>3</sup>/min;
  - a pressure of up to 1 bar; and
  - a frequency covering DC, AC, RF, and the MW ranges.

38. The process according to claim 37, wherein the gas plasma is deposited under the following conditions:

- 5
- a discharge power of up to 500 W;
  - an exposure duration of up to 100 s;
  - a plasma gas flow of up to 100 cm<sup>3</sup>/min;
  - a pressure between 0.001-50 mbar; and
  - a frequency between 2-60 Mhz,
- wherein the discharge power is pulsed to the plasma, the pulse discharges being separated by up to 100 s.

39. The process according to claim 37, wherein the substrate is treated in an after-glow.

40. The process according to claim 38, wherein following pulse discharge, the substrate is after-treated with a pre-selected gas.

41. The process for providing a device according to claim 33, suitable for investigating reactions between interactive bio/chemical species by means of surface plasmon resonance spectroscopy, said process comprising the steps of:

preselecting a free electron metal substrate, which metal substrate is suitable for  
5 allowing investigation by surface plasmon resonance spectroscopy, arranging a pre-selected first  
functional group species on the free electron metal substrate by means of plasma deposition,  
which first functional group species protects the free electron metal substrate from a second  
functional group species whose interaction with the plasma deposited first functional group  
species can be investigated, thereby preventing undesirable interactions between the free electron  
10 metal substrate and the second functional group species, and which first functional group species  
provides a desired functionality for the second functional group species; and

subsequently arranging a second functional group species on the plasma deposited  
layer of the first functional group species, whereafter interaction between the first and second  
functional group species layers, can be investigated by means of surface plasmon resonance  
spectroscopy.

42. The process for providing a device according to claim 33, suitable for  
investigating reactions between interactive bio/chemical species by means of surface plasmon  
resonance spectroscopy, said process comprising the steps of:

preselecting a free electron metal substrate, which metal substrate is suitable for  
5 allowing investigation by surface plasmon resonance spectroscopy, arranging a pre-selected first  
functional group species on the free electron metal substrate by means of plasma deposition,  
which first functional group species protects the free electron metal substrate from a second  
functional group species whose interaction with the plasma deposited first functional group



species can be investigated, thereby preventing undesirable interactions between the free electron  
metal substrate and the second functional group species, and which first functional group species  
provides a desired functionality for the second functional group species.

43. The process according to claim 41, wherein before being exposed to the  
second functional group species, a bio/chemical functional layer is wet chemically arranged on  
the plasma deposited first functional group species layer, said wet chemically arranged functional  
layer being pre-selected for its specificity for the second functional group species and for the  
prevention of non specific interactions with the said second functional group species.

44. A method for investigating the interaction, of pre-determined chemical or  
biological species, comprising the steps of analyzing the interaction between the species arranged  
on a device according to claim 25.

45. A method for investigating the reaction between chemically interactive  
species, wherein the species are exposed to or deposited on the device of claim 25.

46. A method for investigating reactions between interactive bio/chemical  
species, by means of surface plasmon resonances spectroscopy, by the device of claim 25, wherein the  
device comprises a pre-selected free electron metal substrate, and a pre-selected, plasma  
deposited layer arranged on the free electron metal substrate, which plasma deposited

5 functional group species is chosen for its attachment ability to the free electron metal substrate, and for its specificity to further functional group species, whereby the interaction therebetween is investigatable by means of surface plasmon resonance spectroscopy.

47. The method of claim 46, wherein the pre-selected free electron metal substrate consists essentially of gold, and wherein the plasma deposited layer comprises one or more sulphur compounds.

48. The process according to claim 40, wherein the layer arranged by plasma deposition comprises at least one functional group species, and wherein the gas comprises the at least one functional group species.--

**IN THE ABSTRACT:**

After the claims, please insert a page containing the Abstract Of The Disclosure, which is attached hereto as a separately typed page.

**REMARKS**

The specification has been amended to place it into conformance with standard United States Patent practice.

Under date of October 20, 2000, Applicants submitted a substitute sheet containing amended claims 1 and 9 for the above-identified PCT application. Original claims 1-24 and amended claims 1 and 9 have been canceled by this Preliminary Amendment and rewritten as new claims 25-48 to eliminate the multiple dependencies and to bring the claims into conformance with standard United States Patent practice.

An Abstract Of The Disclosure has been added as a separately typed page to be inserted after the claims.

Entry of this Preliminary Amendment is respectfully requested.

Respectfully submitted,

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ORKIN & HANSON, P.C.

By 

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**DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS  
AND PROCESS UTILIZING SUCH DEVICE**

**ABSTRACT OF THE DISCLOSURE**

The invention relates to a device for investigating reactions between interactive species, said device comprising: one or more plasma deposited layers, which layers comprise one or more first pre-selected functional group species, which functional group species are  
5 interactible with a pre-selectable second species.

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DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS  
AND PROCESS UTILIZING SUCH DEVICE

The present invention relates to a device for investigating reactions between interactive chemical and/or biological species, to a process for providing such a device, and to a process for investigating  
5 chemical and/or biological interactions, for example biomolecular interactions, utilizing such a device.

Under chemical and/or biological interactions is also understood chemical and/or biological reactions.

Interactions of specific compounds with solid  
10 surfaces play a crucial role in chemical and biological phenomena and areas including analysis techniques such as RIA's, ELISA's.

For investigating and sensing surface interactions a 'sensitive' surface is required.

15 To study real time surface interactions several techniques are available such as ellipsometry, reflectometry and surface plasmon resonance spectroscopy (SPR). These techniques have in common that they use the reflectance of light, generated by a laser, to analyze  
20 the growth or desintegration of a layer of for instance biological molecules at a surface.

For these techniques, a reflecting surface is necessary. In the case of SPR, a surface comprising a free electron metal for example gold is most frequently  
25 used.

In order to utilize this technique for investigating other interactions, besides the interaction of (bio)molecules with free electron metal surfaces, the free electron surfaces have been modified, for instance,  
30 by the adsorption of bio-molecules such as proteins and the coating thereof with polymeric layers in a solvent cast or spin coat procedures.

Methods have also been developed to provide gold surfaces with specific chemical groups for the

5                   Methods for generating SPR sensor surfaces include arranging an organic surface onto a gold layer by means of a wet chemistry procedure such as solvent casting or spin coating before carrying out a plasma etching procedure.

15 immobilization.

An object of the present invention is to provide an improved device for investigating the

According to a first aspect of the present invention there is provided a device according to any of the claims 1 to 8.

-epoxide, and thiol groups for example.

35 to the present invention.

Since a functional group layer is plasma deposited, control over the deposition thereof, can be accurately carried out, whereby very thin layers can be

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deposited thus providing very sensitive devices, without the need for firstly arranging an organic layer by wet chemical methods on the substrate before any further investigation can be carried out.

5           The process according to the present invention provides a good controllability.

In contrast to processes for providing sensor devices, wherein layers are arranged on a substrate by wet chemical processes which are often time consuming, 10 difficult to carry out, and often result in undesirably thick layers exhibiting a subsequent lack of sensitivity if a great deal of care is not applied, the process according to the present invention is extremely flexible to work and easy to effect and offers a good cost 15 efficiency.

Plasma deposition procedures involve the deposition of organic species from the plasma phase on a substrate. For instance by applying a (volatile) monomer as the gas phase an organic layer the structure of which 20 resembles the corresponding polymer can be deposited. By applying a (volatile) monomer that possesses a chemical functionality a chemical functional polymeric layer can be obtained.

The plasma may be deposited from a monomer 25 preferably being selected from the group consisting essentially of:

- unsaturated monomers; acrylic acid, allyl amine, allyl isocyanate, allyl mercaptan, methacrylic acid, allyl alcohol, allyl acetate, allyl acetic acid, 30 allyl glycidyl ether, 3 allyloxy, 1-2 propanediol, vinyl acetate, acrylic acid halides,
- saturated monomers; alcohols such as methanol, ethanol propanol, acids such as propionic acid, acetic acid and the like, formaldehyde, propionic 35 aldehyde, glutardialdehyde, aminoethane, aminoethanol, ethylene oxide, acetone methane, ethane, propane and the like, whereby the substrate is provided with the corresponding functionality.

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Apart from the plasma deposition of saturated and unsaturated monomers, a functionality can be created in situ, i.e. in the plasma layer, by means of rearrangements of (cyclic) monomers or reaction between a mixture of plasma gases for example, whereafter this in-situ created functionality can be deposited.

Surfaces with a high surface energy, such as metal surfaces in general, may give rise to a rapid surface hydrophobisation due to contamination of the surface by species from its environment. This surface contamination may be disastrous for further surface modification for instance with respect to the stability of the final surface. Therefore this surface contamination should be prevented as much as possible by storing the surfaces in an inert atmosphere and reduction of the time between surface preparation and modification or the surface needs to be cleaned before modification. Plasma etching offers an excellent method for this cleaning. Plasma cleaning is fast and is a clean process in itself since it does not involve the use of organic solvent or substantial amounts of reagents that may have adverse effects on the environment. For the present invention it is advantageous to include an in situ plasma cleaning step of the substrate before the actual modification by plasma deposition.

The plasma deposited layer preferably comprises one or more sulphur compounds, for example thiols, sulfides and/or disulfides, i.e. in the form of mercaptoacetic acid, 2-mercaptopropionic acid, 3-mercaptopropionic acid, 1-mercaptopropanol, 2-mercaptoethanol and the like, preferably diallylsulfide, since, especially when gold is chosen as the substrate, an improved stability is provided.

According to a further aspect of the present invention there is provided a process for investigating the interaction of chemical and/or biological species, for example real time surface interactions, according to claims 14 or 15.



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The invention will now be further clarified by way of the following examples, with reference to figure 1 which graphically shows the immobilization of albumins onto a COOH disk as carried out in example 12.

5

#### Example 1

Preparation of carboxylic acid functional gold surfaces.

Gold coated glass discs (60) were placed in the central position of the plasma reactor which consisted of a glass tubes (l = 150 cm, o= 10 cm) with three electrodes positioned at the outside of the glass tube with the powered electrode in the center and two grounded electrodes positioned at 30 cm distance on both sides of the powered electrode. The electrodes were connected to an RF-generator (13.56 MHz, ENI ACG-3, ENI Power Systems) through a matching network (ENI Matchwork 5) and a matching network control unit (ENI TH-1000, ENI). The generator was controlled by a timer (Apple Ile computer with a time control program).

The reactor was evacuated to a pressure less than 0.001 mbar by a rotary pump (DUO 004 B, Pfeifer) which was equipped with a filter (ONF 025, Pfeifer) to prevent oil back streaming. The pressure was measured by a pressure gauge (Baratron 628A01MDE, MKS Instruments) and read from a display module (PR4000, MKS Instruments). An air flow of 5 sccm/min resulting in a pressure of about 0.12 mbar, was established for 5 minutes after which the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Air flow was controlled by a mass flow controller (type 1259 + PR3000 control unit, MKS Instruments). After the plasma treatment the air flow was continued for 2 minutes and then stopped and an acrylic acid flow was established through the reactor via a direct monomer inlet resulting in a pressure of about 0.03 mbar. To prevent the acrylic acid to reach the pump after leaving the reactor, the acrylic acid flow was bypassed through a cold trap that was cooled with liquid nitrogen. The temperature of the

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acrylic acid in the storage container was room temperature. After two minutes the surfaces were treated with 5 pulses of an acrylic acid plasma at a discharge power of 75 (W), the pulses being separated from each other by 30 seconds of acrylic acid flow through the reactor. After the final pulse the surface were exposed to 2 additional minutes of acrylic acid flow whereupon the acrylic acid flow was stopped and the reactor was brought to atmospheric pressure with air.

10

#### Example 2

##### Preparation of amine functional surfaces

Gold coated glass discs (60) were placed in the plasma reactor as described in example 1. The reactor was evacuated to a pressure of less than 0.05 mbar and an air flow of 5 sccm/min was established for 5 minutes whereupon the discs were treated with a dynamic air plasma (85 W) for 1 minute at the same flow conditions. Then air flow was stopped and an allyl amine flow (0.07 mbar) was established through the reactor the temperature of the monomer storage container was 36°C. After two minutes the surfaces were treated with 10 pulses of an allyl amine plasma at a discharge power of 75 W separated from each other by 10 seconds of allyl amine flow through the reactor. After the final pulse the surfaces were exposed to 2 additional minutes of allyl amine flow after which the allyl amine flow was stopped and the reactor was brought to atmospheric pressure with air.

#### 30 Example 3

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an

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air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air. Following, the surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

Table 1

20	element	surface composition (at%)	
		before rinsing	after rinsing
	C	65.4	62.4
	O	10.3	10.5
	N	17.5	13.6
25	Au	6.8	13.4

**Example 4**

30 Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an argon flow of 5 sccm was established through the reactor. After 2 minutes of argon

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flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

15

**Example 5**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, an allylamine flow at a pressure of 0.095 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to five pulses of 1 second of an allylamine plasma at a discharge power of 170 W, the pulses being separated by ten seconds allylamine flow, followed by five pulses of an allylamine plasma at a discharge power of 85 W, again the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air. Following, the

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surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below.

5 Table 2

element	surface composition (atomic %)
C	62.8
O	9.8
N	20.8
10 Au	6.6

**Example 6**

Gold coated substrates (6) were placed in the  
15 plasma reactor (see example 2) between the cold electrode  
on the gas inlet side of the reactor and the hot  
electrode. The reactor was evacuated to a pressure of  
less than 0.005 mbar and an air flow of 5 sccm was  
established through the reactor. After 2 minutes of air  
20 flow the substrates were treated with a dynamic air  
plasma (5 sccm, 85 W) for 1 minute and subsequently  
exposed to an air flow of 5 sccm for 10 minutes again.  
Then the air flow was stopped and after evacuation of the  
reactor, a mixed flow of allylamine and octadiene (56 v%  
25 allylamine) at a pressure of 0.055 mbar was established  
through the reactor. After two minutes  
allylamine/octadiene flow the substrates were exposed to  
ten pulses of 1 second of an allylamine/octadiene plasma  
at a discharge power of 85 W, the pulses being separated  
30 by ten seconds allylamine/octadiene flow. After the final  
plasma pulse the allylamine/octadiene flow was continued  
for 2 minutes after which the flow was discontinued, the  
reactor was evacuated and subsequently brought to  
atmospheric pressure with air. Following, the surfaces  
35 were analyzed for carbon, oxygen, nitrogen and gold by X-

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ray photo-electron spectroscopy, of which the results are shown in the table below.

Table 3

5	element	surface composition (atomic %)
	C	73.1
	O	7.9
	N	11.7
	Au	7.3

10.

**Example 7**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor, a mixed flow of allylamine and diallylsulfide (66 v% allylamine) at a pressure of 0.065 mbar was established through the reactor. After two minutes allylamine/diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an allylamine/diallylsulfide plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine/diallylsulfide flow. After the final plasma pulse the allylamine/diallylsulfide flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

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Following, the surfaces were analyzed for carbon, oxygen, nitrogen and gold by X-ray photo-electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

Table 4

	element	surface composition (at%)	
		before rinsing	after rinsing
10	C	73.4	68.3
	O	4.4	5.3
	N	8.3	9.0
	S	13.3	16.5
15	Au	0.7	0.9

**Example 8**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of argon flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor, a mixed flow of allylamine and diallylsulfide (66 v% allylamine) at a pressure of 0.065 mbar was established through the reactor. After two minutes allylamine/diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an allylamine/diallylsulfide plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine/diallylsulfide flow. After the final plasma

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pulse the allylamine/diallylsulfide flow was continued for 2 minutes after which the flow was discontinued, the reactor was evacuated and subsequently brought to atmospheric pressure with air.

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#### Example 9

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an air flow of 5 sccm was established through the reactor. After 2 minutes of air flow the substrates were treated with a dynamic air plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an air flow of 5 sccm for 10 minutes again. Then the air flow was stopped and after evacuation of the reactor to a pressure less than 0.005 mbar, a diallylsulfide flow at a pressure of 0.025 mbar was established through the reactor. After two minutes diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an diallylsulfide plasma at a discharge power of 85 W, the pulses being separated from each other by ten seconds diallylsulfide flow. After the final diallylsulfide plasma pulse the diallylsulfide flow was continued for 1 minute after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar. Then an allylamine flow at a pressure of 0.090 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes whereafter the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar and brought to atmospheric pressure with air.

Following, the surfaces were analyzed for carbon, oxygen, nitrogen sulphur and gold by X-ray photo-



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electron spectroscopy, of which the results are shown in the table below. Also surfaces that were rinsed with water for 1 hr and subsequently dried were analyzed by XPS.

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Table 5

element	surface composition (at%)	
	before rinsing	after rinsing
C	69.8	68.3
O	6.9	10.2
N	14.8	12.9
S	8.5	8.6
Au	0.0	0.0

10

15

**Example 10**

Gold coated substrates (6) were placed in the plasma reactor (see example 2) between the cold electrode on the gas inlet side of the reactor and the hot electrode. The reactor was evacuated to a pressure of less than 0.005 mbar and an argon flow of 5 sccm was established through the reactor. After 2 minutes of argon flow the substrates were treated with a dynamic argon plasma (5 sccm, 85 W) for 1 minute and subsequently exposed to an argon flow of 5 sccm for 10 minutes again. Then the argon flow was stopped and after evacuation of the reactor to a pressure less than 0.005 mbar, a diallylsulfide flow at a pressure of 0.025 mbar was established through the reactor. After two minutes diallylsulfide flow the substrates were exposed to ten pulses of 1 second of an diallylsulfide plasma at a discharge power of 85 W, the pulses being separated from each other by ten seconds diallylsulfide flow. After the final diallylsulfide plasma pulse the diallylsulfide flow

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was continued for 1 minute after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar. Then an allylamine flow at a pressure of 0.090 mbar was established through the reactor. After two minutes allylamine flow the substrates were exposed to ten pulses of 1 second of an allylamine plasma at a discharge power of 85 W, the pulses being separated by ten seconds allylamine flow. After the final allylamine plasma pulse the allylamine flow was continued for 2 minutes after which the flow was discontinued and the reactor was evacuated to a pressure less than 0.001 mbar and brought to atmospheric pressure with air.

#### Example 11

##### 15 Coupling of CMD onto amine functionalized gold surfaces.

Carboxymethyl cellulose (100 mg) was dissolved in 10 ml 0.05 M 2-(N-morpholino) ethanesulfonic acid after which 5 mg N-hydroxysuccinimid was added. After complete dissolution of this reagent 20 mg N-(3-dimethylaminopropyl)-N' ethylcarbodiimide was added. After 3 minutes activation, an amine functionalized gold surface was incubated with 1 ml of this carboxymethyl dextran solution for 2,5 hours. Then the surfaces were rinsed with phosphate buffered saline, and water and vacuum dried. The whole immobilization procedure was performed at room temperature.

In this example, carboxymethyl dextran is used as a model compound for chemical functional group containing compounds in general including but not limited to dextrans including carboxymethyl dextran,

carboxymethyl cellulose, mono- di- oligo- and polysaccharides, gum xanthan, carboxylate and amine dendrimers, and mono-, homo- and hetero-functional carboxylate polyethylene glycols and polyethylene oxide, polyethylene imine, polyacrylic acid, polyvinyl alcohol, etc.

The amount of these functional group containing compounds that is immobilized can be controlled by the

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reaction parameters such as reaction time, the concentration of the functional group containing compound and the ratio of coupling agent to functional group containing compound.

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#### Example 12

Immobilization of albumin on a COOH-functionalized sensing device.

A sensor device, that was COOH-functionalized by the plasma deposition method was used for the immobilization of albumin. During the immobilization procedure that was performed at 22.5 °C the surface events were monitored by Surface Plasmon Resonance Spectroscopy of which the results are given in figure 1. After mounting the functionalized sensing device in the SPR apparatus, the sensing surface was incubated with 10 mM HEPES buffer for about 5 minutes. Then the HEPES buffer was exchanged for a EDC (20 mg/ml)-NHS (4 mg/ml) solution in water. After 5 minutes activation the EDC/NHS solution was exchanged for an albumin solution (2 mg/ml in 10 mM HEPES) and an immobilization time of 15 minutes was applied. Then the sensing surface was rinsed with HEPES buffer and the stability of the immobilized albumin in HEPES buffer was monitored for 3 minutes after which the rinsing procedure with HEPES buffer was repeated. To study the stability of the immobilized albumin in 0.1 N HCl the HEPES buffer was replaced by 0.1 HCl and the sensing surface was incubated in this solution for 3 minutes after which 0.1 N HCl was replaced for fresh 0.1 N HCl and the measurement was continued for 3 minutes. Then the surface was rinsed with 0.1 N HEPES buffer again an incubation of the sensing surface was proceeded in this buffer for a final 5 minutes.

The results show that upon activation of the sensing surface with EDC/NHS and subsequent immobilization of albumin and rinsing with HEPES buffer the response increases with about 700 milli-degrees indicating the immobilization of albumin on the COOH-

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functionalized sensing surface. Rinsing of the surface with 0.1 N HCl only resulted in a decrease of the signal of about 30 milli-degrees, showing that the albumin immobilization is very stable.

- 5           The invention is not limited to the above description; the requested rights are rather determined by the following claims.

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## CLAIMS

1. Device for investigating reactions between interactive species, said device comprising:
- one or more plasma deposited layers, which layers comprise one or more first pre-selected functional group species, which functional group species are interactible with a pre-selectable second species.
2. Device according to claim 1 wherein the plasma deposited layer is supported on a substrate.
3. Device according to claims 1 or 2 further comprising a film of a free electron metal, preferably selected from the group consisting essentially of copper, silver, aluminum and gold.
4. Device according to claim 3 wherein the plasma deposited layer is arranged directly on the free electron metal film.
5. Device according to any of the previous claims, wherein the plasma deposited layer, comprises one or more chemical and/or biological functional groups.
6. Device according to claim 5, further comprising one or more wet chemically deposited layer(s), arranged on the plasma deposited layer.
7. Device according to any of the preceding claims wherein the plasma layers comprise one or more amine compounds and/or one or more sulphur compounds, preferably thiols, sulfides and/or disulfides and most preferably being diallyl sulfide.
8. Device according to claim 7, wherein the substrate consists essentially of gold.
9. Process for providing a device according to any of the previous claims, comprising the step of depositing a gas plasma layer onto a pre-selected substrate in order to provide the substrate with a predetermined functionality.

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10. Process according to claim 9 wherein the plasma layer is directly deposited onto the substrate and/or onto a metal film arranged on the substrate.

11. Process according to claims 9 or 10 wherein  
5 plasma is deposited from a monomer/ oligomer/ polymer in gas form, preferably being a monomer, said monomer being saturated, partially saturated or unsaturated.

12. Process according to any of the claims 9-11  
10 wherein the substrate is subjected to a pre-cleaning step comprising pre-treating the substrate by means of a plasma etching step before the plasma deposition step said pre-cleaning step preferably comprising pre-treatment with air plasma.

13. Process according to any of the claims 9-12  
15 wherein the gas plasma is deposited under the following conditions:

- a discharge power of upto 5000 W, preferably upto 500 W,
- an exposure duration of upto 1000 s,  
20 preferably upto 100 s,
- a plasma gas flow of upto 10000 cm<sup>3</sup>/min, preferably upto 100 cm<sup>3</sup>/min,
- a pressure of upto 1 bar, preferably from between 0,001-50 mbar,
- 25 - a frequency covering DC, AC, RF, and the MW, preferably from between 2-60 Mhz.

14. Process according to claim 13 wherein the discharge power is pulsed to the plasma, the pulse discharges being separated by:

- 30 - upto 1000 s preferably upto 100 s.

15. Process according to claims 13 or 14 wherein the substrate is treated in an after-glow.

16. Process according to claims 14-15 wherein following pulse discharge, the substrate is after-treated  
35 with a pre-selected gas, which gas optionally comprises the one or more functional groups which have been plasma deposited.

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17. Process for providing a device according to any of the preceding claims 9-16, suitable for investigating reactions between interactive bio/chemical species by means of surface plasmon resonance

5 spectroscopy, said process comprising the steps of:

- preselecting a free electron metal substrate, which metal substrate is suitable for allowing investigation by surface plasmon resonance spectroscopy, arranging a preselected first functional group species on  
10 the free electron metal substrate by means of plasma deposition, which first functional group species protects the free electron metal substrate from a second functional group species whose interaction with the plasma deposited first functional group species can be  
15 investigated, thereby preventing undesirable interactions between the free electron metal substrate and the second functional group species, and which first functional group species provides a desired functionality for the second functional group species, and

- subsequently arranging a second functional group species on the plasma deposited layer of the first functional group species, whereafter interaction between the first and second functional group species layers, can be investigated by means of surface plasmon resonance  
20 spectroscopy.

18. Process for providing a device according to any of the preceding claims 9-17, suitable for investigating reactions between interactive bio/chemical species by means of surface plasmon resonance

25 spectroscopy, said process comprising the steps of:

- preselecting a free electron metal substrate, preferably being gold, which metal substrate is suitable for allowing investigation by surface plasmon resonance spectroscopy, arranging a preselected first functional  
30 group species on the free electron metal substrate by means of plasma deposition, which functional group species preferably is selected from a sulphur compound, which first functional group species protects the free

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electron metal substrate from a second functional group species whose interaction with the plasma deposited first functional group species can be investigated, thereby preventing undesirable interactions between the free  
5 electron metal substrate and the second functional group species, and which first functional group species provides a desired functionality for the second functional group species.

19. Process according to claim 17 or 18,  
10 wherein before being exposed to the second functional group species, a bio/chemical functional layer is wet chemically arranged on the plasma deposited first functional group species layer, said wet chemically  
15 arranged functional layer being preselected for its specificity for the second functional group species and for the prevention of non specific interactions with the said second functional group species.

20. Device according to claims 1 to 8,  
obtainable according to a process according to any of the  
20 claims 9-19.

21. Process for investigating the interaction,  
for example real time surface interaction, of pre-determined chemical and/or biological species, comprising the steps of analyzing the interaction between the  
25 species arranged on a device according to any of the claims 1 to 8 and/or 20.

22. Use of a device according to any of the claims 1-8, and/or 20 for investigating the reaction between chemically interactive species, and especially  
30- for use in SPR.

23. Use of a device for investigating reactions between interactive bio/chemical species, by means of surface plasmon resins spectroscopy, said device comprising a preselected free electron metal substrate,  
35 and a preselected, plasma deposited layer arranged on the free electron metal substrate, which plasma deposited functional group species is chosen for its attachment ability to the free electron metal substrate, and for its



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specificity to further functional group species, whereby the interaction therebetween is investigatable by means of surface plasmon resonance spectroscopy.

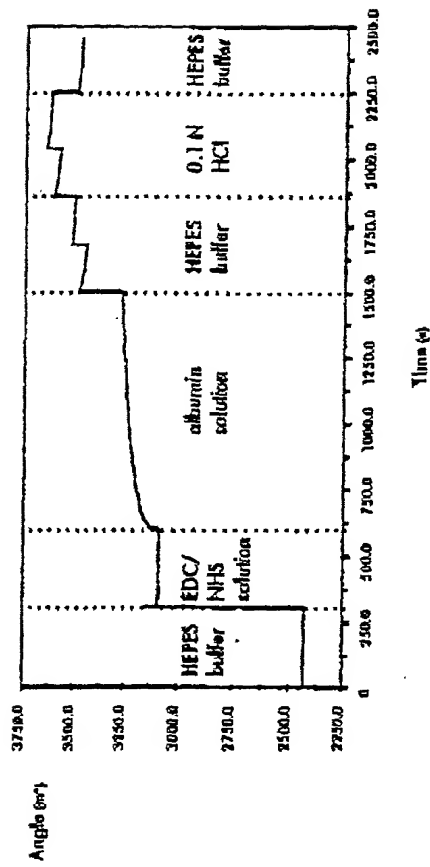
24. Use of a device according to claim 23,  
5 wherein the pre-selected free electron metal substrate consists essentially of gold, and wherein the plasma deposited layer comprises one or more sulphur compounds.

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Figure 1



## Declaration and Power of Attorney For Patent Application

## English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

~~DEVICE FOR INVESTIGATING CHEMICAL INTERACTIONS AND PROCESS UTILIZING SUCH~~  
the specification of which DEVICE

(check one)

☐ is attached hereto.

☒ was filed on August 6, 1999 as PCT/NL99/00504 and as \_\_\_\_\_ as  
Application Serial No. 09/762,779 received 13 February 2001  
and was amended on February 13, 2001  
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

<u>NL 1009871</u> (Number)	<u>The Netherlands</u> (Country)	<u>14 August 1998</u> (Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No
_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes	<input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/NL99/00504

(Application Serial No.)

6 August 1999

(Filing Date)

pending

(Status)

(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. (List name and registration number)

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